P 420

ALTERED RESPONSE TO INTRACELLULAR APPLICATION OF INOSITOL-1,4,5-TRIPHOSPHATE IN CULTURED RETINAL PIGMENT EPITHELIAL CELLS FROM RCS RATS

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Purpose. The retinal degeneration in RCS rats is caused by a malfunction of the retinal pigment epithelium (RPE). Since several lines of evidence point to a disturbed second messenger system in these cells, we compared the effect of intracellular applied inositol-1,4,5-triphosphate (IP3) on RPE cells from RCS and non-dystrophic rats. ***Methods***. IP3 (10µM) was applied to cultured RPE cells from RCS and non-dystrophic control rats via the patch-pipette in the whole-cell configuration of the patch-clamp technique. ***Results***. Intracellular application of IP3 led in both RPE cells from RCS and non-dystrophic rats to activation of voltage-dependent chloride currents. This IP3-induced membrane conductance started to develop 20-40 sec after establishing the whole-cell configuration and produced maximal current amplitudes after 7-10m min. IP3 failed to activate chloride currents under extracellular calcium-free conditions and in cells preincubated for 5min in 1µM thapsigargin. Thus, IP3 led to a release of calcium from cytosolic stores and to an influx of extracellular calcium into the cell which in turn activated a calcium-dependent chloride conductance. Evaluating the rise of chloride currents induced by IP3 expressed as ΔpA/min in per cent to control currents, we found an accelerated rise of chloride currents in RPE cells from RCS rats: +190 ± 9% versus 23 ± 7% (SEM, n = 4) with 1mM calcium in the bath and +580 ± 150% versus +155 ± 15% (SEM, n = 5) with 10mM calcium in the bath. The accelerated rise of chloride currents in RPE cells from RCS rats could be significantly reduced by the calcium channel blocker nifedipine (1µM) to +45 ± 16% (SEM, n = 3). ***Conclusions***. The previously reported increased calcium conductance in RPE cells from RCS rats (Pflüger's Arch. 425: 68-76,1993) leads to a faster influx of calcium into the cells and changes the response of the cells to IP3.

P 421

HUMAN RETINAL PIGMENT EPITHELIUM IN VITRO: IDENTIFICATION OF INTRACELLULAR AND EXTRACELLULAR MATRIX BY INDIRECT IMMUNOFLUORESCENCE.

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Purpose: Retinal pigment epithelial cells (RPE) play an important role in the pathogenesis of Proliferative Vitreoretinopathy (PVR) and humoral and inflammatory factors also are thought to be involved in early PVR formation. Vitreous pigment dusting suggests there are alterations of the blood-ocular barrier during early PVR development and blood-derived factors which may induce PVR (eg chemo-attractants and adhesive proteins like platelet-derived growth factor, transferrin, fibronectin, vitronectin, thrombospondin, factor VIII, thrombin, plasmin, complement factors C1q, C3, C4, immunglobulin G and antigen-antibody-complexes) may gain access to the ocular cavity and influence RPE behaviour. In early culture, RPE cellular antigens tend to be preserved but there are changes in RPE activities which may reflect exposure to blood-derived factors. We continued our investigations of these changes in the cytoskeleton and extracellular matrix of early primary cultured human RPE using indirect immunofluorescence.
Methods: Human postmortem cornea bank eyes, negative for human immunodeficiency virus (HIV) and hepatitis B, which macroscopically showed no morphological abnormality, were used. For establishment of RPE cultures, the anterior segment of the eye was removed. Vitreous and neural retina were seperated and RPE cells were rinsed gently from Bruch´s membrane with culture medium. Isolated cells were plated out in polystyrol lens carrier bottles (Nunc, T.C. Interlab) especially for fluorescence microcopy. After decanting culture medium the bottom of lens carrier bottles was removed, incubated with primary antibody for 60 minutes, rinsed with PBS-Dulbeco, put into antiglobulin (FITC) and prepared for fluorescence microscopy. We used the principle of indirect immunofluorescence (sandwich method): intra- and extracellular matrix antigenic determinants (Ag) were established by specific antibodies building an antigen-antibody-complex in the presence of a fluorescine-conjugated antiglobulin (FITC). We analized micro-filaments (myosin, alpha-actinin, actin), intermediate filaments (cytokeratins, desmin, vimentin), extracellular proteins (collagene II, III, IV, VI) and glycoproteins (laminin, fibronectin).
Results: No immunoreactivity for desmin or actin (stress fibers) was detected, but the RPE stained strongly for myosin, moderately for alpha-actinin and cytokeratin 8, and weakly for vimentin. Strong positivity was observed for fibronectin while the cells were weakly labelled for laminin. Strong immunoreactivity was seen for collagen III and VI, moderate staining for collagen IV and weak labelling for collagen II.
Conclusions: Many cell adhesion molecules are in contact with each other and the RPE cells in the early cultures. The extracellular materials include a solid phase, a soluble phase and a phase at the cell surface and may be important in transferring signals, regulators, carrier proteins and as a moderator of cell migration in early PVR.

P 422

MESSENGER RNA (mRNA) QUANTITATION OF THE RETINAL PIGMENT EPITHELIUM-SPECIFIC PROTEIN RPE65 BY COMPETITIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION DURING THE DEVELOPMENT OF THE RAT RETINA.

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Purpose : In the rat, the retinal pigment epithelium (RPE)-specific protein RPE65 appears at postnatal day (PND) 4, that is 7 days after the RPE has achieved its differentiation, and one to two days before the first photoreceptor outer segments are detectable. As a prerequisite for studies on the transcriptional activation of the RPE65 gene during the development, we quantified the levels of its messenger RNA during the retinal development.
Methods : A rat RPE65 cDNA was obtained by RT-PCR, cloned and sequenced. Using specific cDNA primers, we synthetized a recombinant RPE65 cDNA 105 bp shorter than the wild one. A competitor RNA was obtained from this cDNA by *in vitro* transcription from a primer bearing the T7 RNA polymerase promoter sequence. Quantitative measurements of the RPE65 mRNA levels from the embryonic day (ED) 17 to adulthood were estimated by spiking various amounts of competitor RNA with identical amounts of total RNA from entire eyes in RT-CPR reaction tubes. The quality of the total RNA was controlled by Northern blot.
Results : We found that the RPE65 mRNA is detectable as soon as the ED 17 and that it increases in the following days to reach a value of 0.01pg/100ng of total eye RNA at PND 2. The amount of RPE65 mRNA then slightly varies from 0.003 to 0.01pg/100ng of total eye RNA between PND 2 and PND 5. From PND 5 to PND 10, there is a second increase (100 fold) in the amount of RPE65 mRNA which reaches a plateau thereafter.
Conclusion : There is a two step increase of the RPE65 mRNA levels. This could be due to either variation in messenger stability of transcriptional activation. Wether specific retinal transcription factors are responsible for either or both steps will be assessed in future studies.
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P 423

Messenger RNA Expression study of the retinal pigment epithelium-specific protein RPE65 in the rat retina by *in situ* hybridization.

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Purpose: RPE65 is a retinal pigment epithelium (RPE) protein recently purified. Immunohistological studies have shown that this protein is found exclusively in the RPE. We wanted to determine wether or not the RPE was the unique site of RPE65 synthesis in the eye.
Methods: *In situ* hybridization was performed on tissue sections of frozen rat eyes using two types of non radioactive probes. Digoxigenin-labeled riboprobes were obtained by *in vitro* transcription of the plasmid bovine RPE65 cDNA pPE3. Oligonucleotides deduced from the rat cDNA sequence of RPE65 were synthesized and 3' end-labeled by tailing with digoxigenin-dUTP.
Results: A strong specific staining was found in the RPE from adult rat eyes with the antisense probes, while no staining was visible with the sense probes used as controls. In addition, the RPE65 message was detected in the RPE at earlier developmental stages.
Conclusion: The use of digoxigenin-labeled probes unambiguously demonstrates that RPE65 is exclusively synthesized in the RPE. This specificity of expression and the fact that RPE65 is evolutionary well conserved suggest that this protein could be involved in an important function of the RPE.
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